PATENT COOPERAT I TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 15 June 2001 (15.06.01)	
International application No. PCT/SE00/01808	**Applicant's or agent's file reference** 2001943
International filing date (day/month/year) 19 September 2000 (19.09.00)	**Priority date** (day/month/year) 30 September 1999 (30.09.99)
Applicant ANTONSSON, Per et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

21 March 2001 (21.03.01)

[ ] in a notice effecting later election filed with the International Bureau on:

_____

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Claudio Borton

Telephone No.: (41-22) 338.83.38

TENT COOPERATION TREAT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PC-2001943	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/SE00/01808	International filing date *(day/month/year)* 19.09.2000	Priority date *(day/month/year)* 30.09.1999

International Patent Classification (IPC) or national classification and IPC7
C07K 14/025, C12N 15/86, A61K 48/00

Applicant
ACTIVE BIOTECH AB et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21.03.2001	03.10.2001
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. 08-667 72 88 Telex 17978 PATOREG-S	Authorized officer Viveca Norén/EÖ Telephone No. 08-782 25 00

Form PCT/IPEA/409 (cover sheet) (January 1998)

INTERNATIONAL PRELI[redacted]ARY EXAMINATION REPORT

I[redacted]ional application No.
PCT/SE00/01808

I. Basis of the report

1. With regard to the **elements** of the international application:*

☒ the international application as originally filed

☐ the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the claims:
pages _____, as originally filed
pages _____, as amended (together with any statement) under article 19
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the drawings:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the sequence listing part of the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheet/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2 (c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.*

INTERNATIONAL PRELI[redacted]ARY EXAMINATION REPORT

In[redacted]onal application No.
PCT/SE00/01808

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 21-26

because:

☒ the said international application, or the said claims Nos. 21-26
relate to the following subject matter which does not require an international preliminary examination *(specify)*:

See PCT Rule 67.1.(iv).: Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____
are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. _____ are so inadequately supported
by the description that no meaningful opinion could be formed.

☐ no international search report has been established for said claims Nos. _____

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-20	YES
	Claims		NO
Inventive step (IS)	Claims	1-20	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-20	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The following documents were revealed at the international search:

D1: National Library of Medicine, file Medline, Medline accession no. 95251779, Hines JF et al: "The expressed L1 protein of HPV-1, HPV-6, and HPV-11 display typespecific epitopes with native contormation and reactivity with neutralizing and nonneutralizing antibodies"; & Pathobiology 1994; 62(4):165-71

D2: WO 9915630 A1 (INSERM), 1 April 1999 (01.04.99), page 1, line 28 - line 33

D3: WO 9611272 A2 (MEDIGENE GESELLSCHAFT FüR MOLEKULARBIOLOGISCHE DIAGNOSTIK, THERAPHIE UN TECHNOLOGIE MBH), 18 April 1996 (18.04.96)

D4: WO 9948518 A2 (MEDIGENE AKTIENGESELLSCHAFT), 30 Sept 1999 (30.09.99)

The documents D1-D4 all describe the general state of the art and are not considered to be of any particular relevance to the present invention.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 April 2001 (05.04.2001)

PCT

(10) International Publication Number
WO 01/23422 A1

(51) International Patent Classification[7]: C07K 14/025, C12N 15/86, A61K 48/00

(21) International Application Number: PCT/SE00/01808

(22) International Filing Date:
19 September 2000 (19.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9903534-7 30 September 1999 (30.09.1999) SE

(71) Applicant *(for all designated States except US)*: ACTIVE BIOTECH AB [SE/SE]; Box 724, S-220 07 Lund (SE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: ANTONSSON, Per [SE/SE]; Spårsnögatan 35, S-226 52 Lund (SE). KRISTENSSON, Karin [SE/SE]; Fredsgatan 2, S-222 20 Lund (SE). WALLÉN-ÖHMAN, Marie [SE/SE]; Sotarevägen 10, S-227 30 Lund (SE). DILLNER, Joakim [SE/SE]; Åsevägen 5, S-182 35 Danderyd (SE). LANDO, Peter [SE/SE]; Carl Gustavsväg 26A, S-211 46 Malmö (SE).

(74) Agent: AWAPATENT AB; Box 5117, S-200 71 Malmö (SE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, DZ, EE, EE (utility model), ES, FI, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR (utility model), KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/23422 A1

(54) Title: VACCINE

(57) Abstract: The invention relates to a carrier for introduction of a substance into cells, comprising a major capsid protein L1 of human papillomavirus (HPV-L1 protein) which has been intentionally modified to remove type-specific epitope(s) causing production of neutralising antibodies. The invention also includes an oligo- or polynucleotide coding for said carrier, vaccines comprising said carrier or said oligo- or polynucleotide, as well as methods of using the carrier or the oligo- or polynucleotide in vaccination against infections of human papillomavirus, or against development of consequences of such an infection, or against development of certain cancers.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/01808

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C07K 14/025, C12N 15/86, A61K 48/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C07K, C12N, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	National Library of Medicine, file Medline, Medline accession no. 95251779, Hines JF et al: "The expressed L1 protein of HPV-1, HPV-6, and HPV-11 display typespecific epitopes with native contormation and reactivity with neutralizing and nonneutralizing antibodies"; & Pathobiology 1994; 62(4):165-71 --	1-17
A	WO 9915630 A1 (INSERM), 1 April 1999 (01.04.99), page 1, line 28 - line 33 --	1-26
A	WO 9611272 A2 (MEDIGENE GESELLSCHAFT FüR MOLEKULARBIOLOGISCHE DIAGNOSTIK, THERAPHIE UN TECHNOLOGIE MBH), 18 April 1996 (18.04.96) --	1-26

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 January 2001	17 -01- 2001
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Patrick Andersson/EÖ Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/01808

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9948518 A2 (MEDIGENE AKTIENGESELLSCHAFT), 30 Sept 1999 (30.09.99) -- --------	1-26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE00/01808

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 21-26
because they relate to subject matter not required to be searched by this Authority, namely:

see next sheet

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE00/01808

Claims 21-26 relate to methods of treatment of the human or animal body by surgery or by therapy/ diagnostic methods practised on the human or animal body/Rule 39.1 (iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

INTERNATIONAL SEARCH REPORT

Information on patent family members

04/12/00

International application No.
PCT/SE 00/01808

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9915630 A1	01/04/99	AU 9269898 A	12/04/99
		FR 2768749 A	26/03/99
WO 9611272 A2	18/04/96	AU 4270196 A	02/05/96
		CA 2202090 A	18/04/96
		DE 4435907 A,C	11/04/96
		DE 4447664 C	15/04/99
		EP 0809700 A	03/12/97
		JP 11504801 T	11/05/99
		US 6066324 A	23/05/00
		DE 19526752 A,C	23/01/97
		DE 29521486 U	30/04/97
WO 9948518 A2	30/09/99	AU 3521499 A	18/10/99
		DE 19812941 A	07/10/99

PATENT COOPERATION TREATY

PCT

REC'D 24 OCT 2001
PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference PC-2001943	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/SE00/01808	International filing date *(day/month/year)* 19.09.2000	Priority date *(day/month/year)* 30.09.1999
International Patent Classification (IPC) or national classification and IPC7 C07K 14/025, C12N 15/86, A61K 48/00		
Applicant ACTIVE BIOTECH AB et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21.03.2001	03.10.2001
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Telex 17978 PATOREG-S Facsimile No. 08-667 72 88	Authorized officer Viveca Norén/EÖ Telephone No. 08-782 25 00

Form PCT/IPEA/409 (cover sheet) (January 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE00/01808

I. Basis of the report

1. With regard to the **elements** of the international application:*

☒ the international application as originally filed

☐ the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the claims:
pages _____, as originally filed
pages _____, as amended (together with any statement) under article 19
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the drawings:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the sequence listing part of the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheet/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2 (c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE00/01808

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 21-26

because:

☒ the said international application, or the said claims Nos. 21-26
relate to the following subject matter which does not require an international preliminary examination *(specify)*:

See PCT Rule 67.1.(iv).: Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____
are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. _____ are so inadequately supported
by the description that no meaningful opinion could be formed.

☐ no international search report has been established for said claims Nos. _____

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE00/01808

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-20	YES
	Claims		NO
Inventive step (IS)	Claims	1-20	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-20	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The following documents were revealed at the international search:

D1: National Library of Medicine, file Medline, Medline accession no. 95251779, Hines JF et al: "The expressed L1 protein of HPV-1, HPV-6, and HPV-11 display typespecific epitopes with native contormation and reactivity with neutralizing and nonneutralizing antibodies"; & Pathobiology 1994; 62(4):165-71

D2: WO 9915630 A1 (INSERM), 1 April 1999 (01.04.99), page 1, line 28 - line 33

D3: WO 9611272 A2 (MEDIGENE GESELLSCHAFT FüR MOLEKULARBIOLOGISCHE DIAGNOSTIK, THERAPHIE UN TECHNOLOGIE MBH), 18 April 1996 (18.04.96)

D4: WO 9948518 A2 (MEDIGENE AKTIENGESELLSCHAFT), 30 Sept 1999 (30.09.99)

The documents D1-D4 all describe the general state of the art and are not considered to be of any particular relevance to the present invention.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 April 2001 (05.04.2001)

PCT

(10) International Publication Number
WO 01/23422 A1

(51) International Patent Classification[7]: C07K 14/025, C12N 15/86, A61K 48/00

(21) International Application Number: PCT/SE00/01808

(22) International Filing Date:
19 September 2000 (19.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9903534-7 30 September 1999 (30.09.1999) SE

(71) Applicant *(for all designated States except US)*: **ACTIVE BIOTECH AB** [SE/SE]; Box 724, S-220 07 Lund (SE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ANTONSSON, Per** [SE/SE]; Spårsnögatan 35, S-226 52 Lund (SE). **KRISTENSSON, Karin** [SE/SE]; Fredsgatan 2, S-222 20 Lund (SE). **WALLÉN-ÖHMAN, Marie** [SE/SE]; Sotarevägen 10, S-227 30 Lund (SE). **DILLNER, Joakim** [SE/SE]; Åsevägen 5, S-182 35 Danderyd (SE). **LANDO, Peter** [SE/SE]; Carl Gustavsväg 26A, S-211 46 Malmö (SE).

(74) Agent: **AWAPATENT AB**; Box 5117, S-200 71 Malmö (SE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, DZ, EE, EE (utility model), ES, FI, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR (utility model), KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/23422 A1

(54) Title: VACCINE

(57) Abstract: The invention relates to a carrier for introduction of a substance into cells, comprising a major capsid protein L1 of human papillomavirus (HPV-L1 protein) which has been intentionally modified to remove type-specific epitope(s) causing production of neutralising antibodies. The invention also includes an oligo- or polynucleotide coding for said carrier, vaccines comprising said carrier or said oligo- or polynucleotide, as well as methods of using the carrier or the oligo- or polynucleotide in vaccination against infections of human papillomavirus, or against development of consequences of such an infection, or against development of certain cancers.

VACCINE

FIELD OF THE INVENTION

The present invention relates to a carrier for introduction of substances into cells comprising a modified major capsid protein L1 of human papillomavirus (HPV-L1 protein) devoid of type-specific epitopes causing production of neutralising antibodies. The invention also includes an oligo- or polynucleotide coding for said carrier, vaccines comprising said carrier or said oligo- or polynucleotide, as well as methods of using the carrier or the oligo- or polynucleotide in vaccination against viral, bacterial or parasite infections as well as against development of certain cancers. Especially, infections of human papillomavirus and the development of cancer as a consequence of such infections are recognised.

BACKGROUND OF THE INVENTION

The Human Papillomavirus (HPV) is since long established as the major cause of cervical cancer (1), and has in recent years also been established as a cause of cancers of the penis, vulva, vagina, anus and orofarynx. There also exists indications that the virus may be involved in some cancers of the prostate, esophagus and in other head and neck cancers. HPV vaccine development is therefore a prime priority of preventive cancer research today (2).

The HPVs exist as >100 different types. Although types are defined by genetic homology, the genotypes have hitherto shown a strikingly good concordance with serotypes, i.e. hyperimmune antisera against one type will only neutralise the same type and not other genotypes. Cross-neutralisations have only been reported for certain closely related types and have had titers 2 orders of magnitude less than for the type-specific neutralisation (2,3).

The HPV capsid consists of 72 capsomers each containing 5 copies of the HPV major capsid protein L1. A minor capsid protein, L2, is present in much smaller amounts in the capsid (1:12 compared to the L1 protein) and the location of L2 is uncertain (2).

A number of small viruses express capsid proteins that when expressed self-assemble to form virus-like particles (VLPs) (i.e. particles morphologically similar to virus particles, but lacking the viral genome). The HPV major capsid protein L1 is among the best studied (2). HPV VLPs containing only L1 are morphologically similar to VLPs containing both L1 and L2 (2). Both particles with L1 only and particles with L1/L2 are highly efficient in eliciting a high-titered neutralising antibody response in several animal model systems (rabbits, cows, dogs and rhesus monkeys), even when injected in the absence of adjuvant (2).

Vaccination with papillomavirus VLPs has been shown to be highly efficient for protection, mediated by neutralising antibodies, against subsequent challenge with both cutaneous and mucosal papillomaviruses, but only in a type-specific manner (2). This strong type-specificity is surprising, since the major capsid protein of the HPVs is a highly evolutionarily conserved protein with very few amino acid changes between genetically related, but not cross-neutralising, HPV types.

The most common oncogenic HPVs are HPV16, 18, 31 and 45. HPV16 is found in about 50% of cervical cancers, HPV18 in about 20%, and these four types together correspond to >80% of all cervical cancers. Therefore, a commonly contemplated strategy is to manufacture vaccines containing HPV capsids of the 4 most common HPV types together (2).

Albeit this strategy appears likely to work for achieving significant cancer reduction, it has some distinct disadvantages. The formulation of vaccines containing 4 active components mixed together involves a

substantial additional cost in manufacturing and efficacy testing and quality control of each component.

Furthermore, some 10-20% of cervical cancers are caused by HPV types not included in the presently manufactured vaccine candidates. Apart from the fact that the vaccine could not possibly protect against these types, the possibility also exists that elimination of the 4 most common oncogenic HPV types may cause an increase in the prevalence of the other oncogenic HPV types, thus further diminishing the cancer-preventive gains. This latter scenario is, as predicted from population biology studies, likely to follow if there exists interference between different viral types. Several lines of indirect evidence do indicate that interference between HPV types does exist.

Several other HPV types cause significant morbidity and mortality, most notably HPV 6 and 11 that cause genital condylomas and recurrent respiratory papillomatoses, and HPVs 5 and 8 that cause cutaneous skin-cancers in the immunosuppressed host.In spite of the obvious advantages of broadly cross-reactive vaccines, the possibility to generate a broadly cross-reactive vaccine, by modifying the L1 protein to not contain immunodominant type-specific epitopes, has not been proposed. Several surface exposed and cross-reactive epitopes are exposed on papillomavirus particles (WO 96/33737), but are not immunogenic in the presence of the immunodominant type-specific epitope (4). Therefore, by modifying the L1 to remove immunodominant type-specific epitopes, it should be possible to generate a cross-reactive papillomavirus vaccine, using a modified HPV-L1 protein as a carrier of surface exposed HPV derived antibody epitopes.

Furthermore, VLPs are highly efficient in eliciting a cytotoxic T lymphocyte (CTL) response, and VLP vaccines have been reported to be highly efficacious (through a CD8+cell-dependent mechanism) in preventing and treating transplantable cancers in several mouse models, in spite

of the fact that immunization is made with an exogenous protein (5). The high immunogenicity appears to be due in part to the preservation of an active mechanism for infection of the cell (designated pseudo-infection, as no viral genome is introduced) which results in the capsid protein being processed and presented in the MHC class I presentation pathway (6). VLPs are therefore of general interest from a vaccine biotechnology point of view, since they can be used as a vehicle for efficient immunogenic delivery of any antigen (7).

Efficient immunisation using wild-type HPV VLPs carrying foreign antigens has been demonstrated in several systems, e.g. the MAGE melanoma antigens and human immunodeficiency virus antigens.

A potential problem using VPLs as vehicles for immunogenic delivery is blocking by type-specific neutralising antibodies. In Sweden 16% of the adult population are sero-positive for HPV-16, reflecting the importance of the problem. In addition, therapeutic vaccination is expected to require recurrent treatments, likely to induce a type-specific antibody response towards a wild-type VLP carrier.

Therefore, by modifying the L1 protein to remove type-specific epitopes causing production of neutralising antibodies, as has been described (8), and introduce antibody or T-cell epitopes in this carrier, it should be possible to generate an immunological response towards the introduced peptide, without obstruction from type-specific neutralising antibodies directed towards the carrier itself.

SUMMARY OF THE INVENTION

An object of the present invention is to provide means for preventing and treating viral, bacterial or parasite infections, especially of human papilloma virus, and the development of benign or malign consequences of such infections, as well as means for treating and preventing cancer.

The present invention provides for the use of a modified HPV-L1 protein devoid of type-specific epitopes causing production of neutralising antibodies, as a carrier of a substance into cells. As a result of the modification, this HPV-L1 protein carrier does not induce production of overt neutralising antibodies towards the carrier itself. In an embodiment of the invention, one or more amino acids may be deleted from said protein.

In particular, the invention provides for such an HPV-L1 protein in fusion with a peptide.

The invention also provides for such a carrier which is capable of giving rise to a protective antibody response, which antibody response may be cross-reactive towards two or more serologically defined subtypes of human papillomavirus.

The carrier must be physically coupled, that is fused, to the peptide for which it acts as a carrier, thus creating a fusion protein.

Particularly, peptides derived from HPV proteins and defining linear antibody epitopes and T-cell epitopes are recognised.

There is also envisaged combinations of said carrier with a minor coat protein of human papillomavirus (HPV-L2 protein), native or modified. Also this HPV-L2 protein can itself be fused to one or more further peptides.

The invention also provides for an oligo- or polynucleotide coding for said carrier. The invention makes it possible to create a better basis for eliciting an MHC class I mediated response, i.e. creating cytotoxic T-cells, without giving rise to type-specific neutralising antibodies towards the carrier, or without type-specific neutralising antibodies being present at the start.

It is also possible to use an HPV-L1 protein, modified as described above, as a carrier of oligo- or polynucleotides to cells.

DETAILED DESCRIPTION OF THE INVENTION

In one of its aspect, the invention provides for a carrier for introduction of a substance into cells, comprising a major capsid protein L1 of human papillomavirus (HPV-L1 protein) which has been intentionally modified to remove type-specific epitope(s) causing production of neutralising antibodies. In one preferred embodiment said HPV-L1 protein is in fusion with a peptide.

Preferably, said peptide comprises one or more T-cell epitopes, especially such epitopes derived from tumor, bacterial, parasite, viral or auto-antigens. In another preferred embodiment, said peptide comprises one or more antibody epitopes, such as tumor, bacterial, parasite, viral or auto-antigens, especially papillomavirus antigens.

The carrier can also be combined with a minor capsid protein L2 of human papillomavirus (HPV-L2 protein), which in its turn may be fused to one or more further peptides. These further peptides are e.g. T-cell or antibody epitopes, which may be derived from tumor, bacterial, parasite, viral or auto-antigens.

In a further embodiment the fusion protein is used as a carrier of oligo- or polynucleotides, e.g. such oligo- or polynucleotides which are coding for an antigen or an immunostimulatory (poly)peptide.

In another aspect, the invention provides for an oligo- or polynucleotide coding for the carrier as defined.

In further aspects, the invention provides for vaccines, comprising as an active ingredient a carrier or an oligo- or polynucleotide as defined above.

In further aspects of the invention there is provided methods of preventing or treating viral, bacterial or parasite infections by vaccination with a carrier or an oligo- or polynucleotide as defined above. In a preferred embodiment the infections is caused by papillo-

mavirus.

There is also provided methods of preventing or treating development of benign or malign consequences of human papillomavirus infection by vaccination with a fusion protein or an oligo- or polynucleotide as defined above.

In embodiments of the methods said human papillomavirus infection is warts or laryngeal papillomatosis.

Further aspects of the invention comprise methods of preventing or treating of cancer, including cancer of cervix, penis, vulva, vagina, anus and orofarynx, by vaccination with a fusion protein or an oligo- or polynucleotide as defined above.

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CLAIMS

1. A carrier for introduction of a substance into cells, comprising a major capsid protein L1 of human papillomavirus (HPV-L1 protein) which has been intentionally modified to remove major type-specific epitope(s) causing production of neutralising antibodies.

2. A carrier according to claim 1, wherein one or more amino acids have been deleted.

3. A carrier according to claim 1, wherein said HPV-L1 protein is in fusion with a peptide.

4. A carrier according to claim 3, wherein said peptide comprises one or more T-cell epitopes.

5. A carrier according to claim 4, wherein said one or more T-cell epitopes are derived from a group of antigens comprising tumor, bacterial, parasite, viral or auto-antigens.

6. A carrier according to claim 3, wherein said peptide comprises one or more antibody epitopes.

7. A carrier according to claim 6, wherein said one or more antibody epitopes are derived from a group of antigens comprising tumor, bacterial, parasite, viral or auto-antigens.

8. A carrier according to claim 7, wherein said one or more antibody epitopes are derived from human papillomavirus antigens.

9. A carrier according any one of claims 6-8, capable of giving rise to a protective antibody response.

10. A carrier according to claim 9, wherein said protective antibody response is cross-reactive towards two or more serologically defined subtypes of human papillomaviruses.

11. A carrier according to claim 10, wherein said protective respones is raised against two or more of the group comprising HPV-L1 proteins derived from human papillomavirus implicated in tumor induction.

12. A carrier according to claim 11, wherein said protective antibody response is cross-reactive towards two or more of the group of HPV-L1 proteins comprising L1 proteins of HPV-16, HPV-18, HPV-31 and HPV-45.

13. A carrier according to any one of claims 1-12 in combination with a minor capsid protein L2 of human papillomavirus (HPV-L2 protein).

14. A carrier according to claim 13, wherein said HPV-L2 protein is in fusion with one or more further peptides.

15. A carrier according to claim 14, wherein said one or more further peptides are chosen from a group of antigens comprising tumor, bacterial, parasite, viral and auto-antigens.

16. A carrier according to any one of claims 1-15, in which said substance is an oligo- or polynucleotide.

17. A carrier according to claim 16, whereby said oligo- or polynucleotide is coding for one or more antigens or immunostimulatory (poly)peptides.

18. A vaccine, comprising as an active ingredient a carrier as defined in any one of claims 1-17.

19. A polynucleotide coding for the carrier as defined in any one of claims 1-17.

20. A vaccine, comprising as an active ingredient a polynucleotide as defined in claim 19.

21. A method of preventing or treating viral, bacterial or parasite infections by vaccination with a carrier as defined in any one of claims 1-17.

22. A method according claim 21 of preventing or treating infection of human papillomavirus.

23. A method of preventing or treating development of benign or malign consequences of human papillomavirus infection by vaccination with a carrier as defined in any one of claims 1-17.

24. A method according to claim 23, whereby said human papillomavirus infection is chosen from the group comprising warts and laryngeal papillomatosis.

25. A method of preventing or treating cancer by vaccination with a carrier as defined in any one of claims 1-17.

26. A method according to claim 25, whereby said cancer is chosen from the group comprising cancer of cervix, penis, vulva, vagina, anus and orofarynx.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/01808

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C07K 14/025, C12N 15/86, A61K 48/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C07K, C12N, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	National Library of Medicine, file Medline, Medline accession no. 95251779, Hines JF et al: "The expressed L1 protein of HPV-1, HPV-6, and HPV-11 display typespecific epitopes with native contormation and reactivity with neutralizing and nonneutralizing antibodies"; & Pathobiology 1994; 62(4):165-71 --	1-17
A	WO 9915630 A1 (INSERM), 1 April 1999 (01.04.99), page 1, line 28 - line 33 --	1-26
A	WO 9611272 A2 (MEDIGENE GESELLSCHAFT FüR MOLEKULARBIOLOGISCHE DIAGNOSTIK, THERAPHIE UN TECHNOLOGIE MBH), 18 April 1996 (18.04.96) --	1-26

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 January 2001	17 -01- 2001
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Patrick Anderss n/Eö Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/01808

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9948518 A2 (MEDIGENE AKTIENGESELLSCHAFT), 30 Sept 1999 (30.09.99) -- --------	1-26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE00/01808

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 21-26
because they relate to subject matter not required to be searched by this Authority, namely:

see next sheet

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE00/01808

Claims 21-26 relate to methods of treatment of the human or animal body by surgery or by therapy/ diagnostic methods practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

INTERNATIONAL SEARCH REPORT

Information on patent family members

04/12/00

International application No.
PCT/SE 00/01808

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9915630 A1	01/04/99	AU 9269898 A	12/04/99
		FR 2768749 A	26/03/99
WO 9611272 A2	18/04/96	AU 4270196 A	02/05/96
		CA 2202090 A	18/04/96
		DE 4435907 A,C	11/04/96
		DE 4447664 C	15/04/99
		EP 0809700 A	03/12/97
		JP 11504801 T	11/05/99
		US 6066324 A	23/05/00
		DE 19526752 A,C	23/01/97
		DE 29521486 U	30/04/97
WO 9948518 A2	30/09/99	AU 3521499 A	18/10/99
		DE 19812941 A	07/10/99